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<p>(21) International Application Number: PCT/EP85/00592</p> <p>(22) International Filing Date: 4 November 1985 (04.11.85)</p> <p>(31) Priority Application Number: 5306/84-5</p> <p>(32) Priority Date: 6 November 1984 (06.11.84)</p> <p>(33) Priority Country: CH</p> <p>(71) Applicant (for all designated States except US): BATTELLE MEMORIAL INSTITUTE [US/CH]; 7, route de Drize, CH-1227 Carouge/Geneva (CH).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only) : RINGROSE, Anthony [GB/CH]; 126, chemin de la Montagne, CH-1224 Chêne-Bougeries/Geneva (CH). SUTHERLAND, Ranaid, M. [GB/CH]; 32, rue du 31 décembre, CH-1207 Geneva (CH). DÄHNE, Claus [DE/CH]; 103, avenue du Bois-de-la-Chapelle, CH-1213 Onex/Geneva (CH). PLACE, John, F. [GB/CH]; 42, chemin de la Chevillarde, CH-1208 Geneva (CH).</p>	<p>(74) Agents: DOUSSE, Blasco et al.; 7, route de Drize, CH-1227 Carouge (CH).</p> <p>(81) Designated States: AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, NL (European patent), US.</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: ANALYTICAL APPARATUS FOR OPTICALLY DETERMINING SPECIES IN SOLUTION</p>		
<p>(57) Abstract</p> <p>The instrument comprises as the main optical element a waveguide carrying a reactant specific to an analyte to be determined. Light is injected at one end (proximal end) of the waveguide and is reflected from the other end (distal end) so that it travels forwards and backwards in the guide before being collected. Along its path it interacts with a complex forming on the surface of the guide as the result of the reaction of said reactant with the analyte whereby a light signal is produced of a wavelength different from the exciting signal. The distal end of the waveguide is provided with filter means to block the exciting residual signal wavelength and to freely pass the response signal wavelength thus minimizing background noise.</p>		

ANALYTICAL APPARATUS FOR OPTICALLY DETERMININGSPECIES IN SOLUTION

The present invention concerns an analytical apparatus for optically determining species in solution, more especially for determining bioactive species by reactions of the immunoassay type.

Analytical apparatuses comprising optical fiber probes which can optically monitor the adsorption of chemical species on the fiber core are known. This technique is based on the immersion of a lit optical wave guide, for instance an optical fiber without cladding, in a test solution the refractive index of which is lower than that of the fiber core, whereby an interaction takes place between the evanescent wave component of the beam travelling along the guide and some species in solution to be determined. This approach is particularly interesting for monitoring events in the reaction space in close vicinity to the fiber, i.e. within reach of the evanescent wave component (a few tenths or hundredths of angströms), this being in the case of tests based on the reaction of a first partner in a complexation reaction, this partner being adsorbed or coated on the probe surface, with a second partner dissolved in the sample solution.

Apparatuses suitable for such types of measurements have been recently disclosed in the following references WO84/00817; USP 4,447,546 (HIRSCHFELD et al); GB 2,103,786 (ICI); J.D. ANDRADE et al. Applied optics 23 (11) 1984, 1812-1815.

In the apparatuses of the prior art, the light probe (optical fiber the cladding of which has been at least partly removed) is connected (coupled) by its proximal end to an optical excitation-detection arrangement comporting generally a light source providing an excitation signal of wavelength λ_1 , a beam splitter mirror separator to direct the signal into the proximal end of the fiber and a photodetector oriented at an angle with the source for receiving the test signal returned from the fiber, the beam splitter mirror effecting the separation between incident and returned light signals.

In the preferred approaches of the prior-art, the returned signal

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is a fluorescent signal generated by some component of the species under reaction with a wavelength λ_2 longer than λ_1 . Therefore the beam splitter mirror is preferably a dichroic mirror, i.e. a low pass interference filter with cut-off frequency between the maximum absorption and maximum fluorescence back emission of the fluorophore of interest. This arrangement allows a rather clean separation between the two signals.

Regarding now the probe itself, this is generally constituted by a piece of optical fiber deprived from its normal low refractive index cladding. This probe is generally coated with a film of one of the reactive species involved in an immunotype reaction, e.g. an antibody specific to an antigen to be analyzed. When the lit probe is immersed in the test solution, the antigen reacts on the fiber surface to provide an immunotype complex capable of generating a fluorescence signal when excited by the evanescent wave component; this signal being returned to the detector via the fiber and the optical coupling system. A perturbing signal (noise) may however be produced from the interaction of the excitation signal exiting from the fiber end and the bulk of the analyte and to avoid this drawback the fiber tip has been either blackened or made fully reflective. In the first case, both the incident signal and the forward generated signal are absorbed with consequent decreased response and in the second case both the incident and fluorescent signals are returned to the separator with consequent relative high noise to signal ratio.

The present invention as defined in claim 1 remedies this situation. In order to disclose it in more detail, reference is made to the annexed drawing.

Fig. 1 is a diagrammatic representation of an analytical instrument according to the invention.

Fig. 2 is an enlarged cross-sectional view of a detail, i.e. the coupling means between the probe and the remainder of the optical fiber.

Fig. 3 is a cross-sectional view of the probe tip with one embodiment of means to filter out the incident signal and reflect the test signal.

Fig. 4 is a cross-sectional view of another embodiment of such means.

The apparatus represented on fig. 1 comprises essentially a probe 1 to be immersed in a container 2 containing an analyte solution. The probe 1 is a piece of optical fiber of which the cladding has been removed on nearly its full extent; a small portion designated 1a still has the cladding not removed for mechanical coupling convenience as seen later.

The lower unclad portion of the fiber dipping in the sample liquid is coated with a layer of a reactive material susceptible to bind with the analyte dissolved in the sample to be analyzed in container 2, thus providing a complex on the surface of the guide which will fluoresce at wavelength λ_2 (test signal).

The present apparatus further comprises an optical elements arrangement comprising a light source 3, focussing means 4, a beam separating means 5, a test signal detector 6, a reference signal detector 7 and a section of flexible optical fiber 8 held by positioning means 9 in proper optical position relative to the focussing means for receiving the excitation signal at wavelength λ_1 from source 3 and at a proper angle for ensuring propagation by multiple reflection along the fiber 8. The elements 3 to 8 have been disclosed in the prior art in detail (see the aforementioned references) and need not be further detailed here; suffice to say that the detectors 6 and 7 are normally suitably connected to circuitry for processing the detected signals, amplifying, discriminating and calculating results in the form of electric signals to be displayed as readouts on suitable display means, all such technology being well known from people skilled in the art.

The flexible fiber element 8 is coupled to the probe by coupling means 10 shown in more details on fig. 2. Such means comprise essentially three balls 11 (only two are shown) housed in a frusto-conically shaped recess 12 of a coupler frame 13 and exerting a pinching action on the probe proximal end 14 by virtue of the action of a compression spring 15 resting on an inner upper wall 16 of the recess 12 and pressing against the balls 11 via a plastic ring 17. Probe 1 is therefore easily detachable by pulling off the coupler and interchangeably replaceable by a fresh one when used up after one analytical operation.

In order to return the forward signal λ_2 generated by the fluo-

rescuing of the reactants on the probe surface ("forward" means the direction toward the probe tip) a dichroic mirror is provided at the distal end of fiber 1 as shown in fig. 3. This dichroic mirror comprises three layers of transparent materials 21, 22, 23 having suitable refractive indexes to filter out the incident wavelength λ_1 and freely pass the test signal wavelength λ_2 . The manner in which to select the material with suitable refractive indexes is well known (see for instance Applied optics and optical Engineering, Editor R. KINGSLAKE, Vol. 1, pages 316-322, Academic Press, New-York). The mirror further comprises a metallic fully reflective back layer 24 (for instance of polished aluminum) which will reflect the λ_2 signal backwards into the probe and from there back to the detector 6.

In another embodiment (fig. 4) the distal end of the probe comprises an optical filter of a substance capable of selectively absorbing the signal at wavelength λ_1 and freely passing the test signal at wavelength λ_2 . The arrangement is completed, like in the previous embodiment, by a polished fully reflective metallic mirror 26. Narrow band filters of the type used in this embodiment are well known and available commercially from optical components manufacturers.

The operation of the present apparatus can be described briefly as follows:

The probe 1 bare section is first coated with a reactive component susceptible to selectively bind some kind of analyte to be tested. In addition, in case the obtained complex does not fluoresce by itself, fluorescent labelling is provided in connection with either one or the other of the reactants, being provided however that no fluorescence (or only reduced fluorescence) is produced in the absence of the desired immunoreaction product.

Then the coated probe, after suitable rinsing and drying, is connected to the flexible section 8 by means of the coupler 10. The optical components 3 to 7 and the additional electronics are turned on and the lit probe is immersed into the container 2 containing the analyte to be determined by the specific aforementioned reaction on the probe surface.

When the reaction proceeds a fluorescence test signal of wavelength λ_2 is generated at the probe surface by the interaction of the excitation light evanescent component of wavelength λ_1 and the

analyte complex layer. The backward component of the test signal is directly returned along the fiber toward the detector 6 via fiber 8, and splitter 5 whereas the forward component first reaches the distal end of the fiber where it is back reflected by the mirror 24, 26 after traversing the filtering section (see figs 3 and 4) inserted between the fiber tip and the mirror. Simultaneously, the unwanted excitation component travelling forward is eliminated by absorption. This technique enables to improve the signal to noise ratio at the detector 6 and enables to increase sensitivity with a given light intensity input or to reduce the input energy without sacrificing sensitivity.

The operation of the computation and display circuitry conforms with the disclosures of the prior art and needs not be further developed here.

It is noted that when the present instrument is operated for making immune type analysis, there is no compulsory need to first obtain the liquid to be analyzed as a sample stored in a laboratory container, as for instance when a blood sample is first taken from a patient. Thus, the probe of the present instrument can be directly introduced, in situ, into the bloodstream of the patient or, if not possible, into a by-pass circuit temporarily connected, for instance during surgery. Indeed the probe of the apparatus of the invention is constituted by optical and mechanical elements of sufficiently small size to be used directly in such tests with only a minimum of discomfort for the patient.

C L A I M S

1. An immunoassay analytical instrument comprising an optical fiber probe coupled to an optical system comporting essentially an excitation signal (λ_1) source, a test signal (λ_2) detector and a beam separating means for splitting said test signal at wavelength λ_2 from said excitation signal at wavelength λ_1 , said fiber being coated with a bioreactive reactant capable of specifically bind to an analyte dissolved in a sample of solution to provide an immunoassay type complex, whereby a fluorescent test signal (λ_2) is generated by interaction of said excitation signal (λ_1) injected into said probe and said complex, said test signal being reflectively returned backwards via the mirrored tip of the probe, the coupler and the separating means to the detector to be collected thereby providing the desired analytical information, characterized in that the probe fiber tip is provided with means to block the incident signal λ_1 and selectively pass and return to the detector the test signal λ_2 .

2. The analytical instrument of claim 1, wherein the means to reflect the test signal and to block λ_1 and pass λ_2 consists of a dichroic filtering mirror placed at the tip of the probe.

3. The analytical instrument of claim 1, wherein the means to block λ_1 and pass λ_2 consists in an optical absorption filter interposed between the fiber end and said mirrored tip of the probe.

4. The analytical instrument of claim 1, further comprising between said probe and said system a section of flexible optical fiber, said probe being detachably connected thereto by means of a plug type snap-on connector coupler.

5. The analytical instrument of claim 4, wherein the snap-on connector consists of a hollow plug permanently fitted to one end of said section of flexible fiber, said plug having a distal opening for inserting the free end of the probe and, held in a recess of said plug in communication with said opening, a spring and ball pinching device acting on the proximal bare end of the probe for retaining it in optically coupled position with said flexible section.

6. The use of the analytical instrument of claim 1, for non-invasively analyzing biological liquids in which the probe is contacted in situ with such liquid without requiring that a sample of this

liquid be first taken from a patient.

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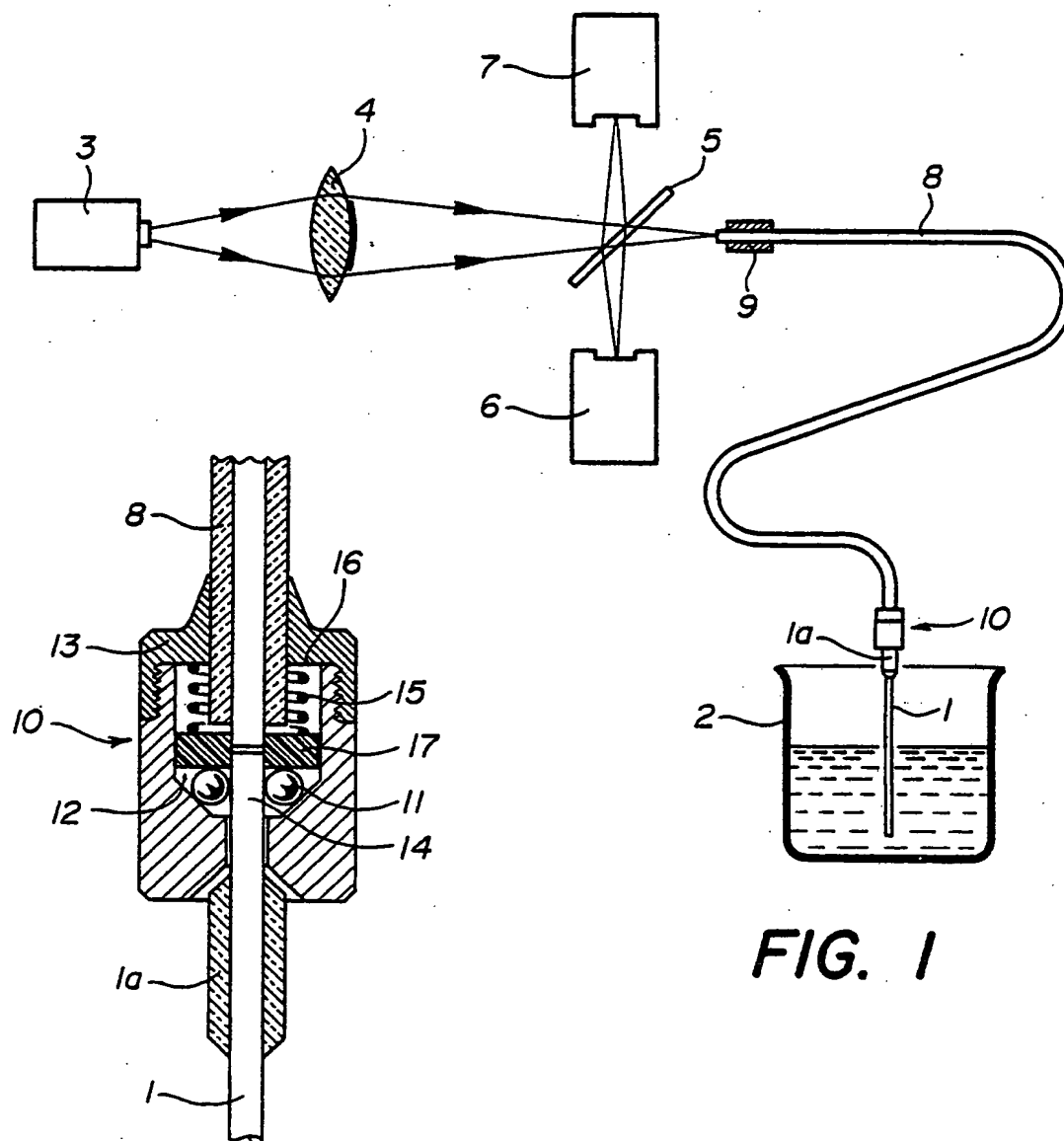


FIG. 1

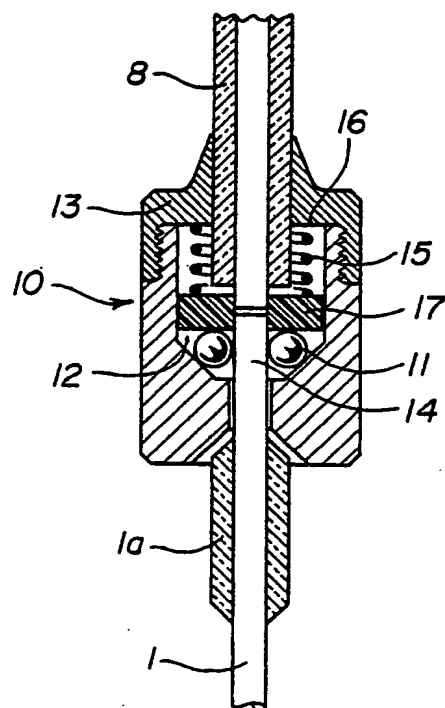


FIG. 2

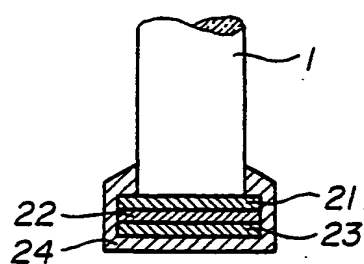


FIG. 3

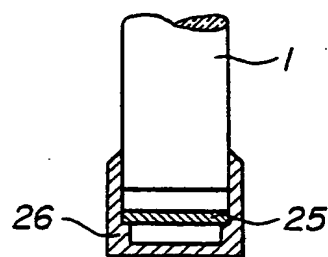


FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 85/00592

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁴ : G 01 N 21/64														
II. FIELDS SEARCHED <div style="text-align: center; margin-top: 5px;">Minimum Documentation Searched ⁷</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%;">Classification System</th> <th style="width: 75%;">Classification Symbols</th> </tr> <tr> <td style="vertical-align: top;">IPC⁴</td> <td> G 01 N 21/64 G 01 N 21/77 G 02 B 6/34 G 01 N 21/85 </td> </tr> </table> <div style="text-align: center; margin-top: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	IPC ⁴	G 01 N 21/64 G 01 N 21/77 G 02 B 6/34 G 01 N 21/85								
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III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%;">Category ⁹</th> <th style="width: 60%;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 30%;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td>WO, A, 84/00817 (M. BLOCK et al.) 1 March 1984, see pages 15-18; figure 1 (cited in the application) --</td> <td style="text-align: center; vertical-align: top;">1,6</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td>EP, A, 0054292 (SIEMENS A.G.) 23 June 1982, see pages 7-9; figures 1-2 --</td> <td style="text-align: center; vertical-align: top;">1</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td>US, A, 3947088 (P. FRENCH) 30 March 1976, see columns 3-4; figures 1-3 -----</td> <td style="text-align: center; vertical-align: top;">4</td> </tr> </table>			Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	Y	WO, A, 84/00817 (M. BLOCK et al.) 1 March 1984, see pages 15-18; figure 1 (cited in the application) --	1,6	Y	EP, A, 0054292 (SIEMENS A.G.) 23 June 1982, see pages 7-9; figures 1-2 --	1	A	US, A, 3947088 (P. FRENCH) 30 March 1976, see columns 3-4; figures 1-3 -----	4
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"d" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">15th February 1986</div> </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">12 MARS 1986</div> </td> </tr> <tr> <td style="width: 50%; padding: 5px;"> International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div> </td> <td style="width: 50%; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;">M. VAN MOL </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">15th February 1986</div>	Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">12 MARS 1986</div>	International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;">M. VAN MOL </div>								
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/EP 85/00592 (SA 11212)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/02/86

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8400817	01/03/84	EP-A- 0115532	15/08/84
EP-A- 0054292	23/06/82	JP-A- 57123497	31/07/82
		US-A- 4356396	26/10/82
US-A- 3947088	30/03/76	US-A- 4029391	14/06/77

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